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Use of the Photo-Electromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI Light Sensitivity

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14. ABSTRACT <u>Purpose:</u> to test the whether photosensitivity (photophobia) after traumatic brain injury (TBI) is due to increased sensitivity of the brainstem trigeminal sensory nucleus, as revealed objectively by an exaggerated photoblink reflex (photo-electromyogram). This will be tested in humans and in a mouse strain genetically engineered to be hypersensitive to calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal nerve function. <u>Scope:</u> objective methods to quantify photo-sensitivity include 1) light evoked potentials (electromyogram) from the blinking and squinting muscles of the forehead 2) the pupil light reflex 3) light evoked changes in sympathetic nerve activity, measured by changes in skin conductance and heart rate. <u>Major Findings (year one):</u> 1) optimization of hardware and software interfaces for recording of the electromyogram (EMG) elicited by light, 2) development of software to simultaneously record and analyze light evoked changes in the EMG, skin conductance, heart rate, and pupil responses. 3) EMG recording from chronically implanted electrodes in the mouse orbicularis muscle was developed using the DSI wireless system of data transmission of biopotentials). <u>Significance:</u> objective testing of photosensitivity in humans and mice will provide new approaches to finding the underlying mechanisms, classification of photosensitivity, diagnosis and monitoring of new treatments.					
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Introduction

Two of the most prevalent problems reported by military personnel following traumatic brain injury (TBI) are photosensitivity and headache. Currently, better means are needed for diagnosing and treating post-traumatic light sensitivity and headache. The goal of this project is to establish a clinically translatable assay of trigeminal function to facilitate diagnosis and treatment of photodynia and headache. The clinically translatable assay will take advantage of a natural brain reflex, the photic-electromyogram (EMG), a reflex contraction of the eyelid muscles in response to light. The photic EMG is modulated by the central trigeminal pain center of the brainstem, which conveys light input to the facial muscles to elicit an eye blink. We hypothesize that the hallmark of patients with photodynia is abnormal sensitization of brainstem trigeminal neurons to light input. This grant's objective is to show that the trigeminal blink reflex to light, as measured by the photic EMG, is a valid surrogate for assessing central trigeminal hypersensitivity as a cause for photodynia and headache, which can be treated. The specific aims of this grant are twofold: 1) to objectively characterize the response of the trigeminal nucleus in humans by recording the photic EMG in normal subjects compared to photosensitive patients and assess treatment with blue blocking lenses, and 2) to examine the response of the trigeminal sensory nucleus in mice by recording the photic EMG in a genetic strain that has trigeminal hypersensitivity and light aversion. The effect of injecting calcitonin gene related peptide (CGRP), the neurotransmitter of trigeminal neurons, and an antagonist, olcegepant, will be used to investigate treatment of photodynia. These studies will establish the foundation for future clinical diagnosis and treatment of photodynia.

BODY – RESEARCH ACCOMPLISHMENTS ASSOCIATED WITH APPROVED STATEMENT OF WORK FOR YEAR 1:

Statement of Work (SOW)

Task 1 (year 1). Optimization of a novel method to objectively assess photosensitivity in humans and mice (months 1-12):

SOW 1a. Optimization of hardware and software interface for human recording of wireless electromyogram (EMG) elicited by light from the procerus and eyelid muscles using “dry” low impedance surface skin electrodes (Sigmed, Inc.) (months 1-4).

Light stimulus source for proposed experiments: In the first year, we received from the manufacturer a super-bright LED TV system that will provide more comfort for subject testing and integration with our visual pathway testing platform. This new super bright large-screen display system (based on a 55” 1920 x 1080 LED TV platform) from DynaScan Technology, Inc. in Irvine, CA (<http://www.dynascanusa.com/>) provides a maximum white level brightness of 5,000 cd/m², compared to 300-700 cd/m² for standard TVs and monitors. This will provide a sufficient dynamic range of stimulus intensities in the white, red and blue chroma that is proposed in the testing of photosensitivity. In the first year, we have integrated our stimulus software with this super-bright monitor so that we are now able to simultaneously record the electrical activity (EMG) of the eyelid and brow muscles in response to light intensities delivered to the patient sitting in front of the monitor screen. We successfully calibrated this monitor and tested out our software, which was written to control the monitor brightness, duration, and color for each visual stimulus. The software has been written to provide an accurate “time stamp” so that a number of different physiologic responses (EMG, pupil response, skin conductance, and heart rate) can be measured in response to the timing of the light stimuli.

Wireless surface EMG “dry” electrode sensing system for recording and transmitting light evoked muscle responses: During the first year, we received delivery of an 8 channel wireless EMG electrode system for the eyelid and eyebrow muscle recording that are mounted on soft cloth. The 8 channels will be dedicated for recording electrical potentials from the skin surface that derive from the electromyogram (EMG), electroretinogram (ERG) and electro-oculogram (EOG) signals in response to light stimuli. This system replaces the wired Biopac modules currently used to collect the same electrophysiological measures. The new system has the advantage of being wireless, no skin preparation or gel is needed at the point of contact with skin, there is a large dynamic range of potential that can be recorded (24 bits, +/- 2.5 volts), and it will allow a much faster set-up time for the proposed experiments. The new type of soft cloth dry electrode for facial sites on sensitive skin will be more comfortable for the patient and easy to apply. We are currently designing a flexible face/eye-mask to hold the electrodes in place. Mark Ginsberg, one of our local jewelry store owners has acquired 3D extruding printers for medical instrumentation applications and will be contracted to refine the design of the electrode holder and will be able to extrude different prototypes to allow us to design and implement a comfortable and effective face/eye mask (similar to the outer rim of swimming goggles) that will hold the electrodes which are mounted on sponge-like material against the skin.

Dr. Poolman has written software code that incorporates a predefined signal analysis sequence of steps that which we will use to process recorded data in real time to measure light induced pupil and EMG responses, as well as skin conductance and ECG responses to light simultaneously. This will allow us to view the processed signal in real time during the recording to troubleshoot any problems that could occur during the testing of subjects and correct them, if needed. He has also supervised our research assistant, Susan Anderson, to incorporate signal analysis software routines in Matlab to derive cumulative EMG signals elicited by light over time and subtract these from baseline EMG signals unrelated to the light stimulus.

For human use approval, we have had a successful pre-screening by the DOD Human Use Coordinator, Brigit Ciccarello, and our local IRB3 (VA and University of Iowa) has reviewed the application and after further modifications, it is in line for approval in the next month. Since we are also utilizing resources from the VA to carry out the grant specific aims, the human use is also being reviewed and approved by the VA Research Committee. The Iowa Animal Use Committee had already approved the proposed experiments on mice earlier in the grant year.

SOW 1b. Integration of software with EMG recording to simultaneously record and analyze skin conductance, beat-to-beat variation in the electrocardiogram (ECG), and pupil responses in humans to increasing levels of light intensity (months 5-8).

Software and hardware integration of light stimulus source and physiologically recorded responses for proposed experiments: In the first year, we have successfully integrated the super-bright LED TV display system into our software control system. This LED/LCD display, as described above, will provide a wide range of visual stimulus possibilities, including chroma (red, blue and white light), timing, and light intensity (up to 5,000 cd/m²) that will satisfy the requirements of our experimental protocol for testing light sensitivity in humans. In addition, we have successfully integrated our SmartEye four camera eye/pupil tracking system into this software and hardware platform so that patient's pupils and EMG can be simultaneously recorded for each light stimulus without having to wear any eye frames with video cameras. This will provide more comfort for the patient during testing and will allow them to respond to the light stimulus in a more natural way, similar to real world visual experiences. In addition, there will be no eye frame physical interference with skin electrode placement needed to record the EMG from the brow and orbicularis oculi muscles. The digital video recorded also allows us to monitor blinking and eye closure, which can be analyzed from the digital video stream collected during each experiment, and will allow us another means of more accurately interpreting responses to light. In addition, the video recording will allow us to analyze in the future for facial features that might show characteristics of an exaggerated response to light, such as squinting. In addition to the recording of pupil size and EMG, we have also completed the integration of the hardware and software for recording of heart rate, finger blood flow (laser Doppler probe) and skin conductance during experiments as measures of sympathetic nerve activity in response to light. It is anticipated that these ancillary physiologic responses may provide additional evidence for an exaggerated autonomic response to light in patients who are pathologically light sensitive.

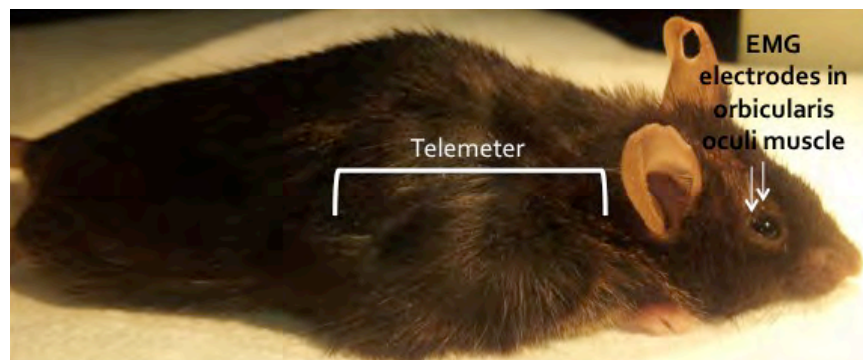
Dr. Poolman has completed writing the software code in the 3rd quarter for designing the light stimulus sequence during experiment runs (which is flexible and easily changed

from a user interface). In addition, the software analyzes in real time the measurements being made in each modality measured and displays this during the experiment. This allows the user to have immediate feedback of the responses being recorded and to make sure there are no data recording problems. Because the software platform was written to time code all measured responses (EMG, pupil size, electroretinogram, electro-oculogram, heart rate, finger blood flow, and skin conductance), all of these are displayed and analyzed in real time. The software is flexible to allow new analysis routines to be implemented as they are optimized and improved upon with real-time display during the course of the experimental run.

During the 3rd quarter, we obtained from the University of Iowa Hospital Information Systems a spreadsheet identifying potential subjects to recruit for the study. The Excel spreadsheet lists patients with a diagnosis of photophobia or photosensitivity and also patients with traumatic brain injury (TBI) from our University of Iowa Hospital database. The database of patients exported into the spreadsheet will be used to identify potential subjects to enroll who are photosensitive from various causes, including TBI. It will also be used to recruit TBI subjects who are not complaining of light sensitivity to be used as one of the control groups proposed.

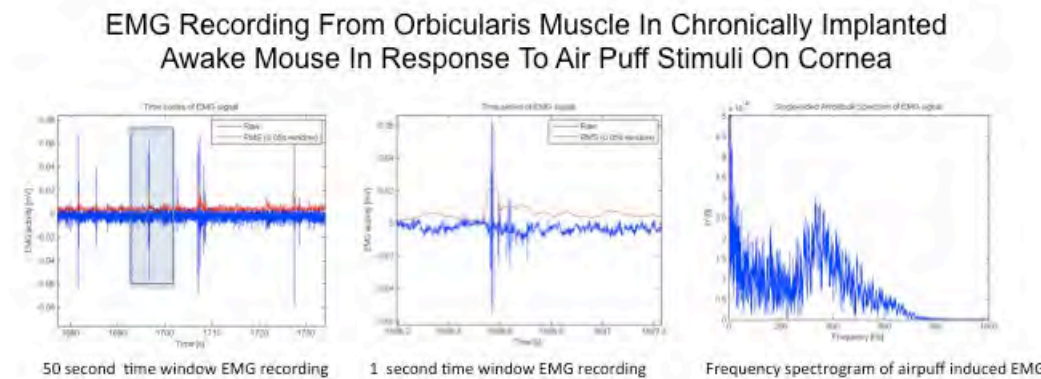
SOW 1c. EMG recording from chronically implanted electrodes in the mouse will be developed using the DSI wireless system of data transmission of biopotentials (months 1-12).

During the first year, we have continued to refine the surgical implantation technique for chronic recording of the EMG from eyelid muscles in response to light and other trigeminal stimuli (i.e. air puff on the cornea) in awake mice. This first involved practice surgeries to implant dummy EMG electrodes into the orbicularis oculi muscle of mice. The transmitters and electrodes were obtained from DSI. All procedures were done under sterile conditions with anesthesia (ketamine/xylazine). In addition, we applied a triple antibiotic ointment to the eyes after surgery to prevent infection. Practice (nonfunctional) transmitters were inserted into a subcutaneous pocket beyond the scapulae on the dorsal flank of three mice. The lead wires were inserted into the superior fibers of the orbicularis oculi using a small needle as a guide. The wires were anchored with glue to the dorsal surface of the skull and the incision was sutured and sealed (see Figure below).



The mouse that has been chronically instrumented with EMG electrodes implanted under the skin into the orbicularis oculi muscles. The lead wires from the electrode pass under the skin and connect to a wireless transmitter that has been implanted on the dorsal area of the mouse. This allows an awake mouse to be monitored for muscle contractions in relation to sensory stimuli such as light and will allow their sensitivity to light to be objectively monitored over time.

In the last quarter, we further refined the implantation procedure. Recently, we confirmed that we have successfully implanted working electrodes for measurement of the orbicularis muscle EMG in mice. The electrodes have been shown to wirelessly transmit the EMG activity in both anesthetized and awake mice that have chronic implantation of the electrodes (see Figure below).



The first tracing shows 50 seconds of recorded EMG activity from an awake, chronically instrumented mouse showing bursts of activity in response to air puff stimulations on the cornea. The middle tracing is an enlargement of one such burst of EMG activity during a 1 second time window shown within the light blue box of the first tracing. The last tracing is a frequency spectrogram of the EMG response from the air puff showing the expected EMG activity from a sensory stimulus.

We have also purchased and received a dedicated small animal computerized pupillometer in the last half of this year to measure the pupil responses to light in the mice. The pupil responses to red, blue and white light will provide a good objective method of assessing the integrity of the afferent retinal and optic nerve input from the mouse eye following the EMG implantation surgery into the orbicularis oculi muscle (blinking muscles).

KEY RESEARCH ACCOMPLISHMENTS (SUMMARY)

- Assembly, calibration, integration and testing of software and hardware for providing time-stamped visual stimuli using a large dynamic range, high luminance flat screen LED/LCD monitor for visual stimuli
- Development and integration of software to simultaneously monitor multiple physiological responses to light stimuli (EMG of brow and orbicularis muscles, skin conductance, laser Doppler finger blood flow, heart rate, pupil responses, and electroretinogram).
- Acquisition of “dry” wireless skin electrode system for monitoring light evoked potentials from the skin surface
- Successful chronic implantation of EMG electrodes into the mouse orbicularis oculi muscle with wireless transmission of EMG in response to stimuli in an awake, unanesthetized mouse.

REPORTABLE OUTCOMES

Presentation of research project to Congressional Briefing in Washington D.C. April 2012 on research in veterans.

Presentation of pilot data as two posters on the Photo-EMG in humans (Carver University of Iowa internal pilot grant) at the Association of Research and Vision in Ophthalmology (ARVO), Ft. Lauderdale, FL May 2012.

Presentation of research project to Blinded Veterans Association, Washington D.C. June 2012

Presentation of invited talk on TBI related photophobia/light sensitivity research at DOD sponsored Military Research Vision Symposium September 2012 (organized by Schepens Eye Research Institute and Harvard/Mass Eye and Ear Infirmary)

Since the first year of this research was restricted to implementation of a hardware and software testing and analysis platform, we have not yet tested human subjects for this funded research (years 2 and 3). Therefore, we do not yet have results of testing to report in the literature.

CONCLUSIONS

The research work that we are carrying out has important implications for the greater public good, in addition to its military relevance. Light sensitivity and migraine headaches following traumatic brain injury are the two most commonly reported symptoms in military personnel exposed to direct trauma to the brain or indirectly from blast injury. Similar symptoms can also occur in the civilian population from TBI resulting from motor vehicle accidents and also from head injury due to contact sports at both the school and professional level. At present there are no biological markers or tests that can be used to objectively diagnose and monitor treatment of photo-sensitivity or migraine headaches. This would be the first research to facilitate investigations of the mechanisms in humans using controlled, photic stimuli with monitoring of physiological reflexes in response to the light stimuli. In order to accomplish this task, it is required that a sophisticated software and hardware integration be in place to accurately measure light evoked reflexes that can be used in research and in a clinical setting. In addition, adding the capability of studying the photic EMG in conscious mice will provide an important scientific platform upon which to use genetic and drug investigations on the mechanism of light sensitivity and migraine and new treatments.

REFERENCES - none

APPENDICES – none

SUPPORTING DATA – all figures including in body of report